-- Brief Description of the Figures

Figure 1. The DNA sequence of the pGT-W insert.

Figure 2. A map of the 9.6kb insert of the lFixII clone isolated from the great tit using pGT-W. pGT1.7 and pGT8 are the two *EcoRI* subclones into which the fragment was divided. The broken line corresponds to the region with absolute sequence identity to the pGT-W insert. The position of the region with identity to the mouse *CHD-1* gene is indicated.

Figure 3. An alignment of 123bp fragment of the great tit (GT) *CHD-W* gene in pGT8 with the autosomal/Z located chicken (C) *CHD-1A* the chicken *CHD-W* gene and bases 3855-3977 of the mouse (M) *CHD-1* gene. An alignment of the deduced amino acid sequence is also given.

B4 Figure 4. The section of pGT8 that hybridized to a female specific fragment of 3.1kb in the chicken. This probe was also used to screen the chicken cDNA library. The hatched line represents the female specific great tit motif shown in Fig. 3.

Figure 5. The complete nucleotide sequence of CHD-1A as defined by the clones Z4, Z6 and Z11. Two asterisks underlie the position where part of the sequence illustrated in Fig7 is spliced onto the 5' or 3' ends of a proportion of the clones isolated. The ATG at nucleotide 228 is the start codon whilst TAA at 5388 is the stop codon.

Figure 6. The strategies used to determine the nucleotide sequence of *CHD-1A* and *CHD-W* given in Fig. 5 and Fig. 8. The top line represents the mouse clone given by (Delmas et al. 1993). The three 'Z' clones of *CHD-1A* and the 'CC4' and 'CC14' clones of *CHD-W* were derived from either a stage 10-12 or a 10 day chick cDNA library respectively. Arrows indicate the direction of sequence determination. Note Z6 actually ran from -227 to 69. These nucleotides were determined and are found in Fig 5

Figure 7. A composite nucleotide sequence and putative translation of the motif that is found spliced to a proportion of the 5' or 3' terminii of *CHD-1* clones or the 3' end of the *CHD-W* clone CC14. The portion attached to the CC14 sequence is incomplete.

134

Figure 8. A partial nucleotide sequence of *CHD-W* as defined by the clones CC4 and CC14.

Figure 9. An alignment of the deduced amino acid sequences of the chicken (C) *CHD-1A* and *CHD-W* with the mouse (M) *CHD-1*. With gaps introduced to maximize alignment they show a sequence identity of 91.6% over 1365 residues. The $ sign indicates start and stop codons. Boxed sections are the chromodomain (C), Helicase (H), and the region containing the DNA binding domain (B) identified by Delmas *et al.*, (1993). A trimer repeat of a basic HSDHR motif is underlined. A* denotes residue identity and . similarity.

Figure 10. An alignment of the deduced amino acid sequences of *CHD-1A* and *CHD-1Y* a putative yeast homologue of the chicken gene identified through a search of the EMBL data base. With

gaps introduced to maximize alignment they show a sequence identity of 37.7% over 1538 residues. | indicates identity and : conservative substitution.

Figure 11. Comparison of 9 chromodomain sequences. Vertical lines indicate the extent of the chromodomain as defined by Paro & Hogness (1991). The top three sequences represent the CHD class of chromodomain to add to the HP1 class and Pc class][;-l08k9ouygytrdevz as defined by Pearce et al. (1992). The first letter of each annotation indicates the animal of origin: C, chicken; M mouse; D, Drosphila; H, human; Y, *S. cerivisiae* whilst the remainder identifies the gene type. The yeast gene is a possible CHD homologue identified by its close identity to the vertebrate forms. * indicates sequence identity within the groups and ^ identity between all nine sequences. _* indicate amino acid residues inside and downstream of the motif that are characteristic of the CHD class chromobox.

B4 Figure 12. Genomic Southern blots of DNA from male and female chickens and lesser black-backed gulls digested with PvuII and probed with a 433bp HindIII/Sac fragment of pGT8 (Fig 4.) at moderate stringency. Hybridization with female linked fragments and fragments common to both sexes can be observed in both species. Numbers give approximate sizes in kilobases.

Figure 13. Genomic Southern blots of DNA from male (M) and female (F) mice, ostrich, chicken, bee-eater and hyacinth macaw probed with the 1335bp insert of CC4 at moderate stringency. Hybridization with mouse and ostrich is with fragments shared by both sexes whilst the non-ratite birds show additional hybridization to female specific fragments. In these latter species, the signal from female linked hybrids is stronger than with autosomal/Z linked fragments indicating that the probe is derived from the W chromosome. Numbers give approximate sizes in kilobases.

Figure 14. The nucleotide sequence of part of a single CHD1 gene isolated from the Mouse and the homologous genes from the Chicken, Hyacinth (A12.3 subclone) and Spix's Macaw all arranged as putative codons. Dashes denote nucleotides shared with the Mouse sequence. The primers designed are shown on the diagram. An arrow head indicates a non-synonymous mutation in the Spix CHD-W. The *DdeI* (CTNAG) and *HaeIII* (GGCC) sites are underlined.

Figure 15. The technique of PCR sex identification in the Spix's Macaw. Semi-nested PCR amplification is carried out on both sexes with the primers P2/P3 then P1/P2 to provide products of identical sizes in both sexes. The products are then cut with restriction enzyme *DdeI* which cuts only the CHD-W product from the female. The cut products are run on a visigel and the difference between the sexes can be visually detected. See Fig 17 for an example.

Figure 16. *DdeI* restricted PCR products demonstrating that remaining wild Spix's Macaw is male. Lane 1. the wild bird 2. negative extraction control 3. known male 4. known female. The larger fragment is of 104 bp and the female W-chromosome specific fragment of 73 bp.

Figure 17. Sex identification in the Marsh Harrier (MH), Chicken (C) and African Marsh Warbler (AMW) carried out using an identical reaction. For each species genomic DNA of male and female birds was subject to PCR with primers P2 and P3 and the product of 110bp is visible in lanes 1 and 2. In lane 3 the entire male PCR product, amplified from *CHD-1A*, has cut into two parts with *HaeIII* (65bp, 45bp). In females, lane 4 this *HaeIII* cut product is also present but the *CHD-W* product remains uncut so the sex can be identified. The 'Kb' lane contains a '1Kb DNA ladder' (BRL), the 'H' lane is PCR reaction with P2 and P3 carried out on human genomic DNA and -ve lane contains a negative PCR reaction.